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DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF GLECAPREVIR AND PIBRENTASAVIR IN DRUG PRODUCT BY UPLC

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ABSTRACT
New Analytical method was developed for the estimation of Glecaprevir and Pibrentasavir in Drug product liquidchromatography. The chromatographic separation was achieved on C18 column (BEH C18 2.1 x 50 mm x 1.7 µm) at 25°C (Ambient) temperature. The separation achieved employing a mobile phase consists of Buffer: Acetonitrile (450:550). The flow rate was 0.3ml/min and ultra violet detector at 260nm. The average retention time for Glecaprevir and Pibrentasavir found to be 0.72 min and 1.27 min. The proposed method was validated as per the guidelines. All validation parameters were within the acceptable range. The assay methods were found to be linear from 50-250ppm for Glecaprevirand 20-100ppm forPibrentasvir.

KEYWORDS: Glecaprevir, Pibrentasvir, Isocratic, UPLC, Mavyret, Method Development & validation.

GLECAPREVIR
Glecaprevir (INN) is a hepatitis C virus (HCV) nonstructural (NS) protein 3/4Aprotease inhibitor that was identified jointly by AbbVie and Enata Pharmaceuticals. It is being developed as a treatment of chronic hepatitis C infection in co-formulation with an HCV NS5A inhibitor pibrentasvir. Together they demonstrated potent antiviral activity against major HCV genotypes and high barriers to resistance in vitro.

Glecaprevir is chemically designated as (3aR,7S,10S,12R,21E,24aR)-7-tert-Butyl-N-{(1R,2R)-2-(difluoromethyl)-1-[(1-methylcyclopropane-1-sulfonyl)carbamoyl] cyclopropyl}-20,20-difluoro-5,8-dioxo-2,3,3a,5,6,7,8,11,12,20,23,24a-dodecahydro-1H,10H-9,12-methanocyclopenta [18,19][1,10,17,3,6] trioxadiazacyclonon adecino[11,12-b] quinoxaline-10-carboxamide.

Its molecular formula is C38H46F4N6O9S and its molecular weight is 838.87 g·mol−1

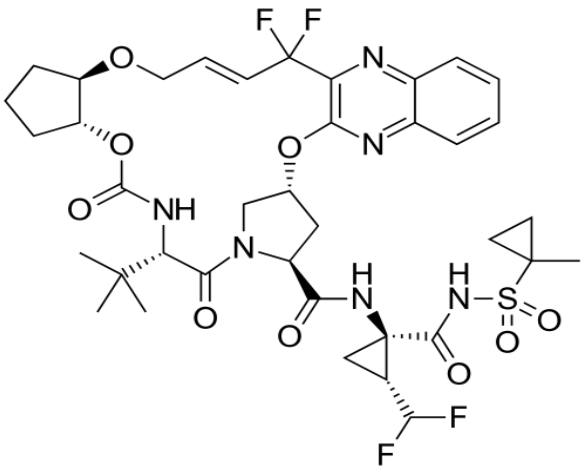

Fig. 1: Structure of Glecaprevir.

PIBRENTASVIR
Pibrentasvir is an antiviral agent.[1] In the United States and Europe, it is approved for use with glecaprevir as the Drug glecaprevir/pibrentasavir (trade name Mavyret in the US and Maviret in the EU) for the treatment of hepatitis C.

Pibrentasvir is chemically designated as Methyl {(2S,3R)-1-[(2S)-2-{5-[(2R,5R)-1-{3,5-difluoro-4-[4-(4-fluorophenyl)-1-piperidinyl] phenyl}-5-(6-fluoro-2-{(2S)-1-[N-methoxycarbonyl)-O-methyl-L-threonyl]-2-

pyrrolidinyl}-1H-benzimidazol-5-yl)-2-yrrolidinyl]-6-fluoro-1H-benzimidazol-2-yl} -1-pyrrolidinyl]-3-methoxy-1-oxo-2-butanyl}carbamate.

Its molecular formula is $C_{57}H_{65}F_5N_{10}O_8$ and its molecular weight is 1,113.20 g·mol−1

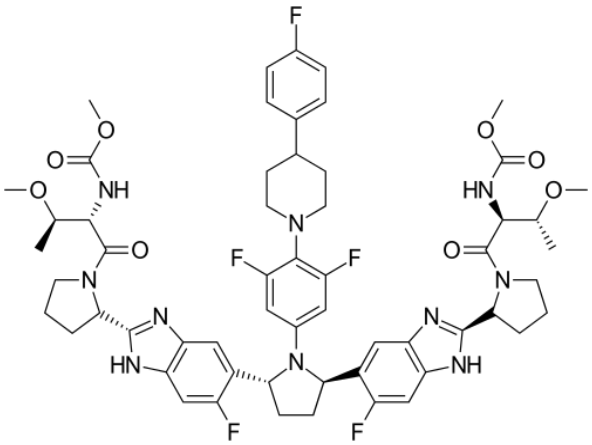

Fig. 2: Structure of Pibrentasvir.

EXPERIMENTAL

Equipment's: The chromatographic technique performed on UPLC waters e2695 Alliance separation module and 2998 PDA with Empower-3 software, Ultrasonic cleaner, analytical balance, Vaccum micro filtration unit with 0.45µ membrane filter and pH meter was used in the study.

Chemicals and Reagents: HPLC-grade Acetonitrile was from Merckreagents pvt ltd. Potassium dihydrogen orthophosphate (AR grade) was from Rankem, Orthophosphoric acid (HPLC grade) from Rankem and Milli Q water.

Chromatographic Conditions: The sample separation was achieved on a (2.1 x 50 mm x 1.7 µm) BEHC18 Column, aided by mobile phase mixture of 0.025MPotassium dihydrogen Phosphate in water pH 3.0: Acetonitrile (450:550). The flow rate was 0.3 ml/ minute and ultra violet detector at 260nm that was filtered and degassed prior to use, Injection volume is 3µl and ambient temperatures.

PREPARATION OF MOBILE PHASE

Buffer Preparation: Weigh and Transfer about 3.4 g of Potassium dihydrogen phosphate into a beaker containing 1000 mL water and adjusting it's the pH of the solution to 3.0 with orthophosphoric acid. Filter through 0.45 µm membrane filter.

Mobile phase: Then added45volumes of buffer, 55 volumes of Acetonitrile mixed well and sonicated for 5 min.

Diluents: Mobile phase.

Preparation of Standard solution

Preparation of Standard stock solution of Glecaprevir

Weigh and transfer accurately about 100.0 mg of Glecaprevir into a 20mL volumetric flask and add 10.0 mL of diluent and sonicate to dissolve and dilute to volume with diluent at room temperature and mix well.

Preparation of Standard stock solution of Pibrentasvir

Weigh and transfer accurately about 40.0 mg of Pibrentasvir into a 20 mL volumetric flask and add 10.0 mL of diluent and sonicate to dissolve and dilute to volume with diluent at room temperature and mix well.

Preparation of Standard solution of Glecaprevir and Pibrentasvir

Transfer each 3.0 mL of Glecaprevir stock solution and 3.0 mL of Pibrentasvir stock solution into 100 mL volumetric flask, dilute to volume with diluent at room temperature and mix well and filter through 0.45 µm PVDF syringe filter. (The resulting solution contains 150 ppm of Glecaprevir and 60 ppm of Pibrentasvir).

Preparation of sample solution

Label claim: Mavyret is a pink colored, film coated, oblong, biconvex -shaped tablet debossed with "NXT" on one side. Each tablet contains 100 mg of Glecaprevir and 40 mg of Pibrentasvir Store at or below 30°C (86°F).

Weigh and powder 10 tablets. Accurately weigh and transfer tablet powder. In this weigh accurately and transfer 286mg of sample (equivalent to 100 mg of Glecaprevir and 40 mg of Pibrentasvir) into a 20 mL volumetric flask add 10.0 mL of diluent and sonicate for 30 minutes to dissolve and dilute to volume with diluent at room temperature and mix well.

Filter the solution through Whatts man NO: 41 filter paper. Discard first 3ml of the filtrate. The filtrate 3.0 mL was quantitatively transferred to a 100-mL volumetric flask with diluent. (The resulting solution contains 150 ppm of Glecaprevir and 60 ppm of Pibrentasvir).

RESULTS AND DISCUSSIONS

Determination of Working Wavelength (λ max): 10 mg of the Glecaprevir and Pibrentasvirstandard drug is taken in a 10 ml volumetric flask and dissolved in Diluent and volume made up to the mark, from this solution pipette out 0.1ml into 10 ml volumetric flask and made upto the mark with the Water to give a concentration of 10 µg/ml. The above prepared solution is scanned in uv between 200-400 nm using Water as blank. The λmax was found to be 260nm.

After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.025MPotassium dihydrogen phosphate in water pH

3.0: Acetonitrile (450:550). The flow rate was 0.3 ml/ minute brought sharp peaks. The chromatogram was shown in Figure-1.

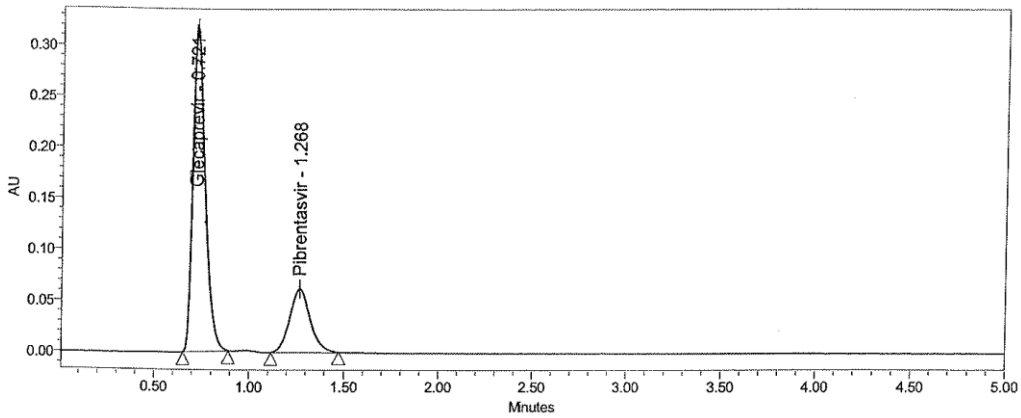

Fig. 3: Chromatogram of Glecaprevir/Pibrentasvir.

METHOD VALIDATION

Linearity

Prepared linearity solutions of Glecaprevir and Pibrentasvir standards at levels of 50,100,150,200,250 ppm of Glecaprevir and 20,40,60,80,100ppm of pibrentasvir, analyzed as per test method and plotted linearity graphs, calculated Square of correlation coefficient.

Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

Table No: 1: Linearity data of Glecaprevir.

Level in ppm	Peak area
50	408856
100	808118
150	1201265
200	1624133
250	2008498
Correlation Coefficient	0.9999

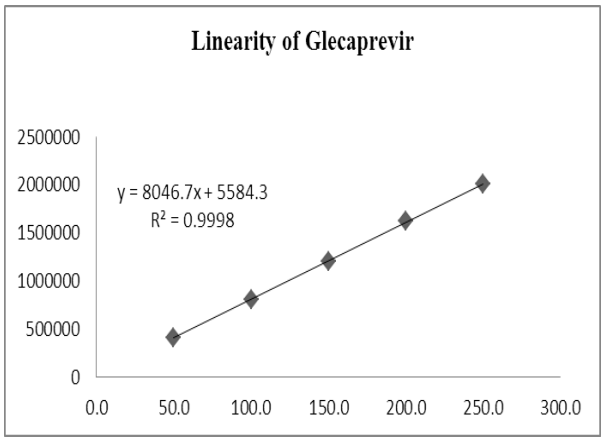

Fig. 4: Linearity (calibration) curve of Glecaprevir.

Table No: 2: Linearity data of Pibrentasvir

Level in ppm	Peak area
20.0	146123
40.0	288525
60.0	425682
80.0	566601
100.0	700030
Correlation Coefficient	0.9999

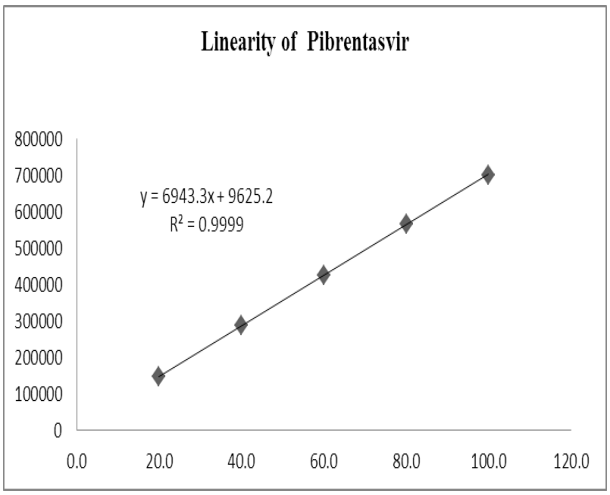

Fig. 5: Linearity (calibration) curve of Pibrentasvir.

Result

A linear relationship between peak areas Versus concentrations was observed for Glecaprevir and Pibrentasvir. Correlation coefficient was 0.9999 and 0.9999 for both Glecaprevir and Pibrentasvir which proves that the method is linear.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on signal to noise ratio. The S/N ratio of Glecaprevir and prebentasavir are given below.

Table no. : LOD and LOQ values Calculated from S/N Ratio.

	Glecaprevir ppm	S/N Ratio	Pibrentasvir ppm	S/N Ratio
LOD	0.09	3:1	0.18	3:1
LOQ	0.3	10:1	0.5	10:1

Result

The detection Limit and Quantification Limit of Glecaprevir and Pibrentasavir are found within the acceptable criteria.

Method precision (repeatability)

The precision of the method was checked by repeated preparation(n=6) of 150ppm of Glecaprevir and 60ppm pibrentasvir without changing the parameter of the proposed chromatographic method.

Table.4: Summary of method precision

S.No.	% Assay	
	Glecaprevir	Pibrentasvir
1	100.2	100.5
2	100.3	100.3
3	100.3	100.2
4	100.4	100.3
5	100.2	100.2
6	100.9	100.2
Average	100.4	100.3
% RSD	0.3	0.1

Result

Results of variability were summarized in the above table. Percentage relative standard deviation (%RSD) was found to be less than 2.0% for both drugs which proves that method is precise**.**

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Glecaprevir and Pibrentasvir by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Glecaprevir and Pibrentasvir. The percentage recovery results obtained are listed in Table 6&7.

Table No. 5: Recovery data of Glecaprevir.

Level	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Average % Recovery	% RSD
50	49.15	49.46	100.6	100.0	0.7
	49.15	48.82	99.3		
	49.15	49.15	100.0		
100	99.62	98.76	99.1	99.5	0.7
	99.62	99.91	100.3		
	99.62	98.74	99.1		
150	148.80	147.63	99.2	99.5	0.3
	148.80	148.56	99.8		
	148.80	148.22	99.6		

Table No.6: Recovery dataof Pibrentasvir.

Level	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Average % Recovery	% RSD
50	20.16	20.28	100.6	100.0	0.6
	20.16	20.05	99.5		
	20.16	20.12	99.8		
100	40.42	40.44	100.1	100.0	0.1
	40.42	40.38	99.9		
	40.42	40.46	100.1		
150	61.08	60.53	99.1	99.9	0.8
	61.08	61.01	99.9		
	61.08	61.44	100.6		

Result

Results of accuracy study are presented in the above table. All the results indicate that the method is highly accurate**.**

Robustness: Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate, detection wavelength and mobile phase variation on assay of the analyte of

interest. Here the detection wavelength varied ±2nm, flow rate was varied ±0.2 ml/min and change in organic composition in the mobile phase about 10%. The results were shown in (Table no.7& Table no. 8).

Table No.7: Results of Glecaprevir.

Parameter	Glecaprevir			
	Rt (min)	% RSD	Theoretical plates	Asymmetry
Flow rate 0.27 mL/min	0.80	0.2	3607	1.1
Flow rate 0.33 mL/min	0.65	0.2	3597	1.1
Wavelength 257 nm	0.72	0.3	3546	1.1
Wavelength 263 nm	0.72	0.1	3538	1.2
Mobile Phase*	0.68	0.2	3600	1.2
Mobile Phase**	0.60	0.2	3603	1.5

Table No.8: Results of Pibrentasvir.

Parameter	Pibrentasvir				
	Rt (min)	% RSD	Theoretical plates	Asymmetry	Resolution
Flow rate 0.27 mL/min	1.37	0.1	3724	1.2	3.3
Flow rate 0.33 mL/min	1.11	0.3	3716	1.1	3.3
Wavelength 257 nm	1.25	0.1	3646	1.1	3.3
Wavelength 263 nm	1.25	0.1	3258	1.2	3.3
Mobile Phase*	1.27	0.1	3681	1.1	3.8
Mobile Phase **	0.89	0.1	3706	1.2	3.3

***Buffer Solution and Acetonitrile in 505:495 v/v ratio**
****Buffer Solution and Acetonitrile in 395:605 v/v ratio**

Result
The results of Robustness of the present method had shown that changes are not significant we can say that the method is Robust.

Ruggedness: Ruggedness was performed by analysing six test preparations different day, different analyst and different column as per the methodology, determined %RSD. The results were shown in Table no.9.

Table No.9: Results of Glecaprevir.

S.No.	% Assay	
	Glecaprevir	Pibrentasvir
1	99.9	100.0
2	99.2	100.4
3	100.4	100.9
4	99.9	100.2
5	99.7	100.0
6	100.5	100.4
Average	99.9	100.3
% RSD	0.5	0.3

Result
The % RSD assay values between two analysts, Different days, Different columns was calculated and found that there was no variability in the test results, this indicates the method was rugged.

Solution Stability
The standard and sample solutions are prepared as per the methodology and analyzed these solutions after 24 hours at 25° C. Calculated similarity factor for standard and % Difference for the test preparations. The results were shown in Table no.10 and 11.

Table No.10: Stability of Standard Solution.

Interval	Similarity Factor	
	Glecaprevir	Pibrentasvir
Initial	NA	NA
After 24 hours	0.99	0.99

Table No.11: Solution Stability of Glecaprevir and Pibrentasavir.

Time	Glecaprevir		Pibrentasavir	
	% Assay	Difference	% Assay	Difference
Initial	100.2	-0.3	100.5	-0.7
After 24 hours	99.9		99.8	

RESULT
The similarity factor for standard under stability against fresh standard was found within acceptable limits and % Assay not deviating more than ±2.0 from initial value.

Forced Degradation Studies
Forced degradation studies were performed to establish the stability, indicating property and specificity of the proposed method.

Table 12: Forced degradation results of Glecaprevir and Pibrentasvir.

Nature of Degradation	Stress Condition	% Degradation for Glecaprevir	% Degradation for Pibrentasavir	%Total Degradation
Acid	0.1 N HCl at 60°C for 24 Hours	8.8%	8.0%	16.8%
Base/Alkali	0.1 N NaOH at 60°C for 24 Hours	7.9%	12.1%	20.0%
Peroxide	30% H_2O_2 at room temperature for 15 minutes	10.3%	8.5%	18.8%
Dry heat	Drug substance heated at 110° C for 3 hours	1.2%	0.0%	1.2%
Photolytic	UV Chambers at 250-watt hours/m2 for 24 hours and white light 1200 wh/sq meter	1.4%	1.0%	2.4%

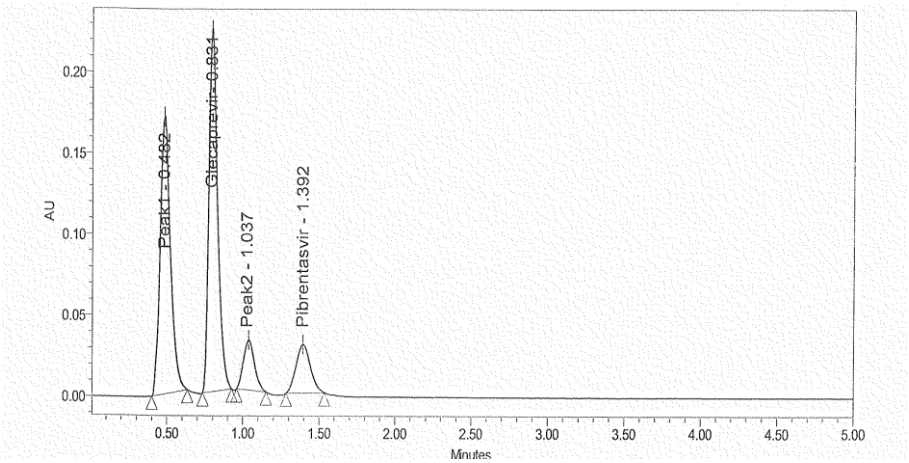

Fig.: 6: Typical Chromatogram of Degradation sample.

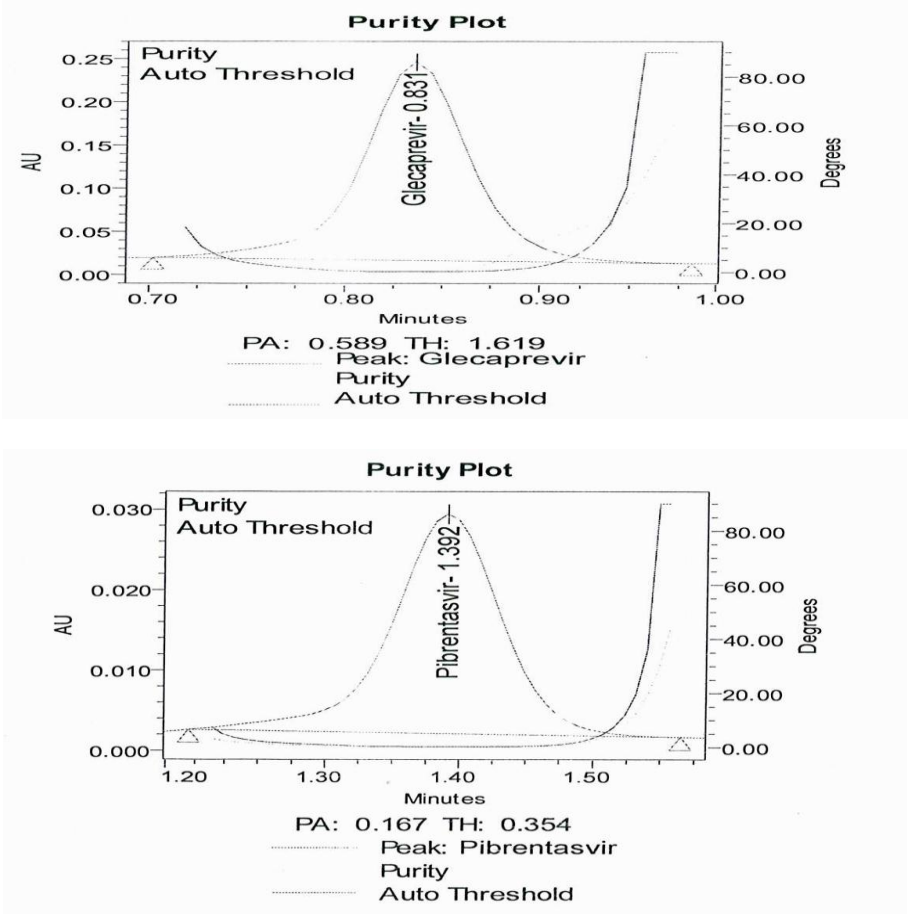

Fig.: 7: Purity plot of Degradation sample.

Table No.13: Summary of Glecaprevir.

S.No	Parameter	Result	Acceptance criteria
1	System suitability Theoretical plates Asymmetry Retention time %RSD	 3593 1.2 0.72 0.1	 Not less than 2000 Not more than 2.0 Not more than 2.0
2	Specificity	Specific	Specific
3	Method precision(%RSD)	0.3	Not more than 2.0%
4	Linearity Range(ppm) Correlation coefficient(r)	50-250 0.9999	 Not less than 0.990
5	Limit of Detection (S/N Ratio) Limit of Quantification (S/N Ratio)	3:1 10:1	S/N between 3 or 2:1 S/N is 10:1
6	Accuracy (Mean % recovery) 50% 100% 150%	 100.0 99.5 99.5	 97 - 103%
7	Robustness	All the system suitability parameters are within the limits.	All the system suitability parameters must be within the limits.
8	Solution stability Similarity Factor (For standard After 24hrs) %Assay Difference (For sample solution)	 0.99 -0.3	 0.98-1.02 ±2.0 from initial value

*RSD = Relative standard deviation

Table No.14: Summary of Pibrentasvir.

S.No	Parameter	Result	Acceptance criteria
1	System suitability Theoretical plates Asymmetry Retention time %RSD	 3618 1.1 1.27 0.3	 Not less than 2000 Not more than 2.0 Not more than 2.0
2	Specificity	Specific	Specific
3	Method precision(%RSD)	0.1	Not more than 2.0%
4	Linearity Range(ppm) Correlation coefficient(r)	20-100 0.9999	Not less than 0.990
5	Limit of Detection (S/N Ratio) Limit of Quantification (S/N Ratio)	3:1 10:1	S/N between 3 or 2:1 S/N is 10:1
6	Accuracy (Mean % recovery) 50% 100% 150%	 100.0 100.0 99.9	 97 - 103%
7	Robustness	All the system suitability parameters are within the limits.	All the system suitability parameters must be within the limits
8	Solution stability Similarity Factor (For standard After 24hrs) %Assay Difference (For sample solution)	 0.99 -0.7	 0.98-1.02 ±2.0 from initial value

*RSD = Relative standard deviation

CONCLUSION

From the above experimental results it was concluded that, this newly developed method for the simultaneous estimation of GLECAPREVIR and PIBRENTASVIR was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research

institutions, quality control department in meant in industries, approved testing laboratories.

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